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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/827,854	04/05/2001	Vassilis I. Zannis	07180/004003	6635

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EXAMINER	
NGUYEN, QUANG	

ART UNIT	PAPER NUMBER
1633	

DATE MAILED: 09/19/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/827,854	ZANNIS ET AL.	
	Examiner	Art Unit	
	Quang Nguyen, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 July 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *30,31,33,34,36,37,43,44,46,47,51,53-62 and 64-72* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *30,31,33,34,36,37,43,44,46,47,51,53-62 and 64-72* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 7/27/05 has been entered.

Amended claims 30-31, 33-34, 36-37, 43-44, 46-47, 51, 53-62 and 64-72 are pending in the present application, and they are examined on the merits herein with SEQ ID NO:15 (apoE3) and adenoviral vector as the previously elected species.

Claim Objections

Claim 31 is objected to under 37 CFR 1.75 as being a substantial duplicate of claim 30. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). This is because the nucleic acid in the expression vector of claim 30 must be operably linked to a promoter, otherwise how else can the polypeptide is expressed?

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Amended claims 30-31, 33-34, 36-37, 43-44, 46-47, 51, 53-62 and 64-72 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

A method of lowering cholesterol in a mammal that lacks an endogenous normally functioning apoE gene, said method comprises intravascularly administering to said mammal a recombinant replication defective adenovirus comprising a nucleic acid sequence encoding a secreted polypeptide comprising amino acid residues 1-185 of SEQ ID NO:2, wherein said nucleic acid sequence does not encode amino acids 260-299 of SEQ ID NO:2 and said polypeptide, when expressed and secreted in said mammal, lowers the total serum cholesterol without inducing hypertriglyceridemia,

does not reasonably provide enablement for a method of lowering cholesterol in any mammal without inducing hypertriglyceridemia by intravascularly administering to said mammal any other recombinant expression vectors as broadly claimed. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims. ***This is a modified rejection with a new ground of rejection.***

The factors to be considered in the determination of an enabling disclosure have been summarized as the quantity of experimentation necessary, the amount of direction or guidance presented, the state of the prior art, the relative skill of those in the art, the

predictability or unpredictability of the art and the breadth of the claims. *Ex parte Forman*, (230 USPQ 546 (Bd Pat. Appl & Unt, 1986); *In re Wands*, 858 F.2d 731, 8 USPQ 2d 1400 (Fed. Cir. 1988)).

The specification teaches by exemplification showing the construction of recombinant adenoviruses expressing secreted apoE4 and various secreted truncated forms of apoE4 (e.g., apoE4-185, apoE4-202, apoE4-229, EpoE4-259). In an apoE-deficient mouse model, the recombinant adenoviruses were injected intravenously through the tail vein and the effects of full-length apoE4 and its various truncated forms on cholesterol and triglyceride homeostasis were evaluated. Applicants showed that an insignificant reduction of the mouse cholesterol level and a severely induced hypertriglyceridemia were observed in apoE-deficient mice treated with full-length apoE4-adenovirus, whereas reduced levels of cholesterol without the induction of hypertriglyceridemia were obtained in animals treated with recombinant adenoviruses expressing the aforementioned truncated forms of apoE4. Applicants further demonstrated that overexpression of either full-length apoE3 or apoE4 is sufficient to induce combined hyperlipedimia (high cholesterol and triglyceride levels) in normal C57BL6 mice, whereas an overexpression of apoE4-202 has no detectable effect on triglyceride levels of the C57BL6 mice.

The above evidence has been noted and considered. However, the evidence is not reasonably extrapolated to the instant broadly claimed invention for the following reasons.

(a) The breadth of the claims

The claims encompass a method of lowering cholesterol in any mammal (e.g., a mammal lacking an endogenous normally functioning apoE gene, a mammal lacking an endogenous normally functioning LDL receptor or a mammal having any lipid disorder) without inducing hypertriglyceridemia, said method comprises intravascularly administering to said mammal any expression vector (e.g., viral or non-viral vector) comprising a nucleic acid sequence encoding any polypeptide (not necessarily a secreted polypeptide) comprising a region of at least 150 amino acids having at least 90% sequence identity to any corresponding region of amino acid residues 1-185 of SEQ ID NO:2, wherein said nucleic acid does not encode amino acids 260-299 of SEQ ID NO:2.; with human apoE3 polypeptide having SEQ ID NO:15 and adenoviral vector as the elected species. It is also noted

(b) The state and the unpredictability of the art

The nature of the instant claims falls within the realm of gene therapy. At the effective filing date of the present application (4/6/2000), the state of the gene therapy art was and still remains unpredictable with respect to the attainment of desired therapeutic effects, for this instance lowering the total serum cholesterol level without inducing hypertriglyceridemia in any mammal, including a mammal having any lipid disorder, as evidenced by the reviews of Verma et al. (Nature 389:239-242, 1997; IDS), Dang et al. (Clin. Cancer Res. 5:471-474, 1999), Romano et al. (Stem Cells 18:19-39, 2000) and Kawashiri et al. (Curr. Control Trials Cardiovasc. Med. 1:120-127, 2000). Dang et al. stated "Although significant progress has been achieved in our understanding of the limitations of gene therapy by suboptimal vectors, host

immunological responses to the vectors, and the lack of long term stable expression, the major challenge that limits clinical translation remains in achieving efficient gene delivery to target tissues" (page 474, col. 2, last paragraph). Romano et al. stated "The potential therapeutic applications of gene transfer technology are enormous. However, the effectiveness of gene therapy programs is still questioned" (see abstract), and "Despite the latest progress reported in the area of vector design, research strategies still have to tackle critically important issues, such as further improvement of gene transfer technology, especially for *in vivo* gene delivery applications, regulation and control of the transgene expression post-cell transduction, and a variety of complex safety matters. These three main issues are to some extent intertwined and pose severe limitations on the applications of gene transfer technology in therapy" (page 21, col. 1, first paragraph). In October 2000, Kawashiri et al. still stated "Somatic gene therapy is a viable approach to the therapy of several lipid disorders for which therapies are currently inadequate" and "The next decade is therefore likely to witness several clinical trials of gene therapy for lipid disorders" (see Conclusion section, page 125). Kypreos et al. (FASEB J. 15:1598-1600, 2001) also stated "One major parameter in successful gene therapy approaches is **gene dosage** and **expression levels**....The inability of the truncated apoE forms that lack all or part of the carboxyl-terminal 260-299 region to induce hypertriglyceridemia, coupled with their intact ability to clear cholesterol, makes them attractive candidates in future gene therapy applications to correct remnant removal disorders" (page 1600, col. 2, last paragraph). Thus, it is clear

that at the effective filing date of the present application gene therapy for the treatment of any lipid disorder was still immature and not routine.

Additionally, at the effective filing date of the present application (4/6/2000) although substantial evidence in the prior art as well as the findings of the present invention suggested or indicated that ApoE functioned **to decrease cholesterol while increasing triglyceride levels** (see references cited on page 6, lines 4-25 of the instant specification), the findings of Tsukamoto et al. (J. Clin. Invest. 100:107-114, 1997; Cited previously) and Kashyap et al. (J. Clin. Invest. 96:1612-1620, 1995) indicated that under their experimental conditions the infection of ApoE-deficient mice with a recombinant adenovirus capable of expressing a mature, full-length human ApoE (299 amino acid residues) resulted in **a reduction in the plasma total cholesterol level without an induction of hypertriglyceridemia**. Thus, at the effective filing date of the present application it was apparent that the biological activity of the ApoE proteins to maintain cholesterol and triglyceride homeostasis *in vivo*, at least in ApoE-deficient mice, was still unpredictable, let alone in any mammal particularly one with any lipid disorder or disease.

The unpredictability of the biological activity of the ApoE proteins to maintain cholesterol and triglyceride homeostasis *in vivo* is further supported by the results of Yoshida et al. (Circulation 104:2820-2825, 2001) that showed that ApoE-deficient mice receiving apoE-/- bone marrow cells that express human apoE3 or apoE2 or apoEcys142 have cholesterol levels increasing with age and the cholesterol levels are not affected by apoE expression (see abstract). Interestingly, the lesion in male apoE3

mice was 40% smaller than that of control mice, while the lesion of apoE2 mice was similar to that of control mice and apoEcys142 mice showed an unexpected and significant increase in lesion size. It is further noted that ApoE2 differs from apoE3 by having a cysteine instead of an arginine at residue 158; and apoEcys142 contains 2 amino acid substitutions: an arginine substitution for cysteine at residue 142 and an arginine for cysteine substitution at residue 112.

(c) The amount of direction or guidance presented

Apart from the exemplification using an apoE-deficient mouse model with recombinant adenoviruses expressing secreted apoE4 or one of the secreted truncated apoE variants apoE4-185, apoE4-202, apoE4-229, EpoE4-259, the instant specification fails to provide sufficient guidance for a skilled artisan on how to lower the total serum cholesterol level without inducing hypertriglyceridemia in other mammals with other recombinant expression vectors comprising a broadly claimed nucleic acid sequence, and particularly the nucleic acid may not even encode a signal peptide that is necessary for the secretion of the encoded apoE variants. This is because it is uncertain whether the desired therapeutic effects could be obtained in any other treated mammals including a mammal having any lipid disorder or disease by administering the recombinant adenovirus expressing any of the disclosed truncated apoE4, let alone using an expression vector comprising a broadly claimed nucleic acid sequence. According to a review by Kawashiri et al., the ApoE knockout mouse model is not a representative mouse model for any lipid disorder, at best it serves as a model for ApoE deficiency and familial dysbetalipoproteinemia. With regard to the animal model used in

gene therapy, Orkin et al. (Report for The Third Meeting of The NIH Investment in Research on Gene Therapy, August, 1995) note that unfortunately, mouse models often do not faithfully mimic the relevant human conditions (Orkin, page 11, second full paragraph), and that animal models are not satisfactory for studying many important disorders, including cystic fibrosis, various cancers, and AIDS. Additionally, Dijk et al. (J. Lipid Res. 40:336-344, 1999, IDS) demonstrated that in LDL receptor-deficient mice both low and high expression of apoE3 via adenovirus-mediated gene transfer **did not result in a reduction of hypercholesterolemina**, and severe hypertriglyceridemia was always induced (see abstract and Fig. 1). Linton et al. (J. Clin. Invest. 101:1726-1736, 1998) also demonstrated that reconstitution of macrophage apoE in apoE(-/-)/low density lipoprotein receptor LDLR(-/-) mice **had no effect on serum lipid and lipoprotein concentrations**, although it produces plasma apoE levels up to 16-fold higher than in C57BL/6 controls (see abstract and Table 1). More recently, Yoshida et al. (Circulation 104:2820-2825, 2001) demonstrated that ApoE-deficient mice receiving apoE-/- bone marrow cells that express human apoE3 or apoE2 or apoEcys142 have cholesterol levels increasing with age and **the cholesterol levels are not affected by apoE expression** (see abstract). There is also no evidence in the instant specification indicating or suggesting that any non-secreted apoE variants of the present invention are capable of exerting any biological effect in a treated mammal, let alone the desired therapeutic effects contemplated by Applicants. Thus, in light of the state of the relevant art and particularly the unpredictability of the *in vivo* biological activity of the apoE proteins as discussed above, coupled with the lack of sufficient guidance provided

by the present application, it would have required undue experimentation for a skilled artisan to make and use the methods as broadly claimed.

With respect to the breadth of claims encompassing the utilization of a nucleic acid sequence encoding any polypeptide comprising a region of at least 150 amino acids having at least 90% sequence identity to any corresponding region of amino acid residues 1-185 of SEQ ID NO:2, as long as the nucleic acid does not encode amino acids 260-299 of SEQ ID NO:2. to lower the total serum cholesterol level without inducing hypertriglyceridemia in a treated mammal, the instant specification is not enabled for the full breadth of the claims. This is because apart from the exemplification showing that the amino-terminal 1-185 residues of human apoE4 are sufficient for binding to lipoprotein remnants to an extent that promotes their efficient clearance in apoE-deficient mice, whereas the carboxyl-terminal 260-299 region of human apoE4 contributes to hypertriglyceridemia, the instant specification fails to provide sufficient guidance for a skilled artisan on which modification(s), for example deletion, insertion or substitution, in which combination(s), and at which amino acid residues in **"the corresponding region"** of amino acid residues 1-185 of SEQ ID NO:2, so that the modified polypeptide still possesses the desired properties (lowering the total serum cholesterol level without inducing hypertriglyceridemia in a mammal). As is well recognized in the art, any modification (even a "conservative" substitution) to a critical structural region of a protein is likely to significantly alter its functional properties. Guo et al. (PNAS 101:9205-9210, 2004) estimated that only about a third of single amino acid changes would completely inactivate the average protein and increasing the

number of substitutions additively increases the probability that the protein would be inactivated and that specific proteins may be more or less tolerant to changes (see the entire article). Furthermore, there is no evidence of record or in the prior art at the effective filing date of the present application that any truncated apoE polypeptide, including truncated apoE3, that is 184 amino acid residues in length or less is still capable of lowering total serum cholesterol level *in vivo*, let alone for an encoded 150 amino acid residue-polypeptide as encompassed by the breadth of the claims. Therefore, in light of the unpredictability of the biological activity of the ApoE proteins for lowering the total serum cholesterol level without inducing hypertriglyceridemia *in vivo* as discussed extensively above, and particularly in view of **the variable isoform-specific effects of ApoE** reported by Yoshida et al. in ApoE deficient mice; coupled with the lack of sufficient guidance provided by the present disclosure it would have required undue experimentation for a skilled artisan to make and use the methods as broadly claimed.

With respect to the breadth of claims encompassing the utilization of any recombinant expression vector, e.g., recombinant viral and non-viral vector, apart from the exemplification there is no evidence of record indicating that any other recombinant expression vectors are also effective in delivering the encoded polypeptide intravascularly to a treated mammal to yield the desired therapeutic effects. Particularly, in light of the unpredictability in obtaining therapeutic effects via gene therapy as discussed extensively above. Interestingly, it is noted that Athanasopoulos et al. (Human Molecular Genetics 9:2545-2551, 2000) have shown that intramuscular

plasmid injection in apoE-/- mice with plasmid vectors expressing allelic human apoE2 or apoE3 isoforms **did not result in any reduction of plasma cholesterol nor in plasma triglycerides** compared to control injected mice (see Table 1, and abstract). Thus, with the lack of sufficient guidance provided by the present disclosure, it would have required undue experimentation for a skilled artisan in the art to make and use the methods as broadly claimed.

As set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the are; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

Accordingly, due to the lack of sufficient guidance provided by the specification regarding to the issues discussed above, the unpredictability of the gene therapy as well as the relevant art on the biological activity of apoE protein in lowering the total serum cholesterol level without inducing hypertriglyceridemia, and the breadth of the instant claims, it would have required undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

Response to Arguments

Applicants' arguments related in part to the above rejection in the Amendment filed on 7/27/05 (pages 9-19) have been fully considered, but they are not found

persuasive. Additionally, the Declaration under 37 CFR 1.132 filed 7/27/05 is insufficient to overcome the rejection of claims 30-31, 33-34, 36-37, 43-44, 46-47, 51, 53-62 and 64-72 based upon insufficiency of disclosure under 35 U.S.C 112, first paragraph, as set forth above, for the following reasons:

1. With respect to the breadth of the encoded polypeptide and the treated mammal in the claimed methods, Applicants argue basically that when a polypeptide having a region corresponding to, or having biological activity equivalent to, the N-terminal region of apoE (excluding the C-terminal amino acids 260-299) is expressed in a subject, the polypeptide will reduce the serum cholesterol level in the subject without inducing triglyceridemia, regardless whether the subject lacks an endogenous, normally functioning apoE, normally functioning LDL receptor or has a lipid disorder. Additionally, this biological activity has been demonstrated in an accepted mouse model that is predictive of the biological activity of the truncated apoE polypeptide in a human as evidenced by the Declaration of Dr. Zannis.

Please note that apart from the exemplification showing that the amino-terminal 1-185 residues of human apoE4 are sufficient for binding to lipoprotein remnants to an extent that promotes their efficient clearance in apoE-deficient mice, whereas the carboxyl-terminal 260-299 region of human apoE4 contributes to hypertriglyceridemia, the instant specification fails to provide sufficient guidance for a skilled artisan on which modification(s), for example deletion, insertion or substitution, in which combination(s), and at which amino acid residues in **"the corresponding region"** of amino acid residues 1-185 of SEQ ID NO:2, so that the modified polypeptide still possesses the

desired properties (lowering the total serum cholesterol level without inducing hypertriglyceridemia in a mammal). As is well recognized in the art, any modification (even a "conservative" substitution) to a critical structural region of a protein is likely to significantly alter its functional properties. Guo et al. (PNAS 101:9205-9210, 2004) estimated that only about a third of single amino acid changes would completely inactivate the average protein and increasing the number of substitutions additively increases the probability that the protein would be inactivated and that specific proteins may be more or less tolerant to changes (see the entire article). There is also no evidence of record or in the prior art at the effective filing date of the present application that any truncated apoE polypeptide, including truncated apoE3, that is 184 amino acid residues in length or less is still capable of lowering total serum cholesterol level *in vivo*, let alone for an encoded 150 amino acid residue-polypeptide as encompassed by the breadth of the claims. To further support the unpredictability of the instant broadly claimed invention, Yoshida et al. (Circulation 104:2820-2825, 2001) showed that ApoE-deficient mice that received apoE-/- bone marrow cells that express human apoE3 or apoE2 or apoEcys142, have cholesterol levels increasing with age and the cholesterol levels are not affected by apoE expression (see abstract). Additionally, Dijk et al. (J. Lipid Res. 40:336-344, 1999, IDS) demonstrated that in LDL receptor-deficient mice both low and high expression of apoE3 via adenovirus-mediated gene transfer **did not result in a reduction of hypercholesterolemina**, and severe hypertriglyceridemia was always induced (see abstract and Fig. 1). Linton et al. (J. Clin. Invest. 101:1726-1736, 1998) also demonstrated that reconstitution of macrophage apoE in apoE(-/-)/low

density lipoprotein receptor LDLR(-/-) mice **had no effect on serum lipid and lipoprotein concentrations**, although it produces plasma apoE levels up to 16-fold higher than in C57BL/6 controls (see abstract and Table 1). Furthermore, a review by Kawashiri et al. indicated that ApoE knockout mouse model is not a representative mouse model for any lipid disorder, at best it serves as a model for ApoE deficiency and familial dysbetalipoproteinemia.

The Declaration of Dr. Zannis does not provide any objective evidence that supports the full enabled breadth of the methods as claimed, especially in light of the specific teachings cited above.

2. With respect to the breadth of an expression vector in the claimed methods, Applicants argue basically that because expression vectors, and their use in promoting the expression of polypeptides in host cells, are well known in the art, the scope of the present claims are fully enabled.

Applicants' argument may be valid for *in vitro* or cell culture expression systems, but not *in vivo*, particularly in a subject in which therapeutic effects are desired. It is still unpredictable in obtaining effective *in vivo* transgene expression levels that yield desired therapeutic effects as evidenced by the numerous gene therapy arts cited above. As an example, Athanasopoulos et al. (Human Molecular Genetics 9:2545-2551, 2000) have shown that intramuscular plasmid injection in apoE-/- mice with plasmid vectors expressing allelic human apoE2 or apoE3 isoforms **did not result in any reduction of plasma cholesterol nor in plasma triglycerides** compared to control injected mice (see Table 1, and abstract).

3. With respect to the general unpredictability in the art, Applicants argue that Applicants are not required **to disclose every species even in an unpredictable art** by citing *In re Vaeck*, 947 F.2d 488, 496 (Fed. Cir. 1991), and that none of the references cited by the Examiner suggest that gene therapy does not work or is completely unpredictable.

Please note that as set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> **That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill** in the are; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

Additionally, *In re Shokal*, 113 USPQ 283 (CCPA 1957):

> It appears to be well settled that a single species can rarely, if ever, afford sufficient support for a generic claim. In re Soll, 25 C.C.P.A. (Patents) 1309, 97 F.2d 623, 38 USPQ 189; In re Wahlforss et al., 28 C.C.P.A. (Patents) 867, 117 F.2d 270, 48 USPQ 397. The decisions do not however fix any definite number of species which will establish completion of a generic invention and it seems evident therefrom that such number will vary, depending on the circumstances of particular cases. Thus, in the case of small genus such as the halogens, consisting of four species, a reduction to practice of three, or perhaps even two, might serve to complete the generic invention, while in the case of a genus comprising hundreds of species, a considerably larger number of reductions to practice would probably be necessary.

Furthermore, the courts have also stated that reasonable correlation must exist between scope of exclusive right to patent application and scope of enablement set forth in the patent application (27 USPQ2d 1662 *Ex parte Maizel.*).

The cited references clearly demonstrate the unpredictability for obtaining the instant broadly claimed invention, and once again paragraphs 4-6 in the Declaration of

Dr. Zannis do not provide any objective evidence that supports the full enabled breadth of the methods as claimed

4. With respect to the cited reference of Dijk et al. (J. Lipid Res. 40:336-344, 1999), Applicants argue that the teachings of Dijk et al. are not inconsistent with Applicants' data with respect to the hypertriglyceridemia-inducing effects of ApoE overexpression, and that Figure 1B clearly showed that serum cholesterol levels were significantly reduced relative to serum cholesterol levels in the control apoE-/-,LDLR-/- mice at eight days following infection with an adenoviral vector expessing full length apoE.

It is noted that the reference of Dijk et al. was cited to demonstrate overexpression of exogenous apoE3 does not necessarily lead to a lower level of total serum cholesterol in LDL receptor-deficient mice, given the fact that hypertriglyceridemia was also expected because of the utilization of a full-length apoE3. Although Figure 1B showed a transient lower serum cholesterol level in apoE-/-,LDLR-/- mice treated with an adenoviral vector expessing full length apoE, it only occurred at a specific gene dosage of 2×10^9 PFU and on the eighth day before the cholesterol level was elevated up to that of the control. Given this observation, Djik et al. still concluded that "Surprisingly, a very high level of APOE expression also did not result in a reduction of hypercholesterolemia in Apoe-/-,LDLR-/-mice, despite very high levels of circulating apoE" (see abstract). Thus, it is apparent that the observed transient drop in serum cholesterol level in treated apoE-/-,LDLR-/- mice occurring under specific conditions was not significant. Otherwise, why would the authors make such a conclusion?

5. With respect to the teachings of Linton et al., Applicants again argue that the reference does not prove the non-enablement of the present claims.

Linton et al. (J. Clin. Invest. 101:1726-1736, 1998) demonstrated that reconstitution of macrophage apoE in apoE(-/-)/low density lipoprotein receptor LDLR(-/-) mice **had no effect on serum lipid and lipoprotein concentrations**, although it produces plasma apoE levels up to 16-fold higher than in C57BL/6 controls (see abstract and Table 1). This indicates that the exogenous supply of apoE is irrelevant or has no effect on the serum lipid and lipoprotein concentrations in apoE(-/-)/low density lipoprotein receptor LDLR(-/-) mice.

6. With respect to the Yoshida reference, Applicants argue that Yoshida et al. merely disclose that the expression of wild-type apoE3 in apoE-/- mice protects against the development of atherosclerotic lesions, while the expression of apoE2 and a variant apoEcys142 do not provide protection. Applicants further argue that the reference actually supports the enablement of the present claims because it confirms that an apoE polypeptide can be successfully and predictably expressed following administration using an adenoviral vector and that one skilled in the art can easily identify apoE polypeptides, using routine experimentation, that do not provide the required effect.

Please note that Yoshida et al. (Circulation 104:2820-2825, 2001) showed that ApoE-deficient mice receiving apoE-/- bone marrow cells that express human apoE3 or apoE2 or apoEcys142 have cholesterol levels increasing with age and **the cholesterol levels are not affected by apoE expression** (see abstract). The results indicate

clearly the unpredictability of the biological activity of ApoE proteins, at least to maintain cholesterol homeostasis *in vivo*, a therapeutic effect sought by the claimed methods. Additionally, the reference clearly demonstrates that a single amino acid substitution between ApoE2 and ApoE3 proteins can have a significant effect in their biological acitivity *in vivo*, let alone for the breadth of the encoded polypeptide to be utilized in the claimed methods.

7. With respect to the cited Kawashiri and Orkin references, Applicants presented the same arguments as those already presented in the Amendment filed on 10/26/04 (page16). These arguments are respectfully found to be unpersuasive for the same reasons already set forth in the Office Action mailed on 1/25/05 (pages 9-10).

Accordingly, claims 30-31, 33-34, 36-37, 43-44, 46-47, 51, 53-62 and 64-72 are rejected for the reasons set forth above.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 30-31, 33-34, 36-37, 43-44, 46-47, 51, 53-55, 57, 59, 61 and 72 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. ***This is a new ground of rejection.***

In claim 30 and its dependent claims, it is unclear what is encompassed by the phrase "a region of at least 150 amino acids having at least 90% sequence identity to

the corresponding region of amino acid residues 1-185 of SEQ ID NO:2", and therefore it renders the claims indefinite. Which is "the corresponding region" of amino acid residues 1-185 of SEQ ID NO:2? Additionally, how does a region of 150 amino acids correspond to the region of 185 amino acids? Clarification is requested because the metes and bounds of the claims are not clearly determined.

Conclusions

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's mentor, David Guzo, Ph.D., may be reached at (571) 272-0767, or SPE, Dave Nguyen, at (571) 272-0731.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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DAVID GUZO
PRIMARY EXAMINER